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Bacterial cellulose production from a single sugar α-linked glucuronic acid-based oligosaccharide

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ABSTRACT

A single sugar α-linked glucuronic acid-based oligosaccharide (SSGO), which is a by-product produced during bacterial cellulose (BC) synthesis by *Gluconacetobacter hansenii*, was evaluated as an additive to synthetic medium during BC production. We assessed the ability of the SSGO to serve as an alternative carbon source to glucose for BC production. To improve the BC yield from glucose by *G. hansenii* PJK, SSGO was added to a chemically defined medium (CDM). In the presence of 1% SSGO, the amount of BC produced was 10.5 g/L after 10 days of cultivation, whereas in the absence of SSGO, only 7.4 g/L of BC was produced. These results indicate that SSGO can be used as an alternative carbon source for BC production. Moreover, the addition of SSGO to the CDM increased the diameter of the BC fibers, which improved its tensile strength and water release properties.

1. Introduction

Cellulose, which is the most abundant biopolymer in nature, is a homopolymer of β-(1,4) glucose. Cellulose is a major component of higher plants and can also be produced by some bacterial strains [1]. Bacterial cellulose (BC) does not contain lignin and hemicelluloses and is the purest form of cellulose [2]; thus, BC has several advantages over plant cellulose. Specifically, BC has a high water holding capacity, high mechanical strength, elasticity, high crystallinity, biocompatibility, polyfunctionality, and hydrophilicity [2–4]. BC is used in speaker diaphragms, artificial blood vessels, face masks, wound dressings, severe body burns, treatment for skin injuries, chronic ulcers, and skin grafts [4], as well as a viscous substance and temporary skin substitute [2,5]. BC is also used in drug delivery systems [6] as an immobilization medium for enzymes [7], as well as a conductive material for various applications [8,9]. Moreover, it also has potential applications in bone tissue engineering [10].

Due to the numerous potential applications of BC, the compound must be efficiently, yet cost-effectively, produced in high yields to achieve commercialization. Several reports have described various attempts to modify and improve BC production. For example, several studies have explored the use of different media with various carbon and nitrogen sources and have attempted to optimize the bacterial culture conditions [11–13]. Additional studies have also assessed the effects of aeration [14,15] and reactor design [16–18]. Hwang et al. [15] studied the effect of pH on cell growth and cellulose production and found that increasing the pH from 4 to 5.5 during BC production resulted in increased productivity compared to a constant pH. Numerous studies have shown that the *Acetobacter* species produce maximal BC yields at pH values between 3.5 and 6 [14]. Moreover, culture medium supplements, such as agar [19], lignosulfonate [20], and plastic composite supports [16], also increase BC production. Several studies have also assessed using the waste from beer fermentation broth as a substitute for CDM during BC production in static culture [21,22]. Modified reactor surfaces have also been reported to impact BC production in static conditions [23]. Similarly, an increased BC productivity was obtained with the addition of 1.0% (v/v) ethanol to CDM with an agitated culture [24]; an additional study showed that optimizing the rotational speed of an impeller in an agitated culture eliminated cellulose non-producing cells (*Cel*$^-$) that arose via spontaneous mutation [1]. A bioreactor equipped with a spin filter and centrifugal and inclined centrifugal impellers was also developed for the same purpose [25,26].

BC biosynthesis involves several complex biochemical reactions and numerous key enzymes [27,28]. The proposed biosynthetic pathways [28,29] suggest that glucose is not entirely converted to BC and that some glucose is transformed into water-soluble oligomers. Chao et al. [30] suggested that by-product formation should be minimized to increase BC production. Several studies have shown that the culture medium composition and fermentation conditions significantly affect the chemical structure and composition of microbial polysaccharides, including BC [31–33].

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In the present study, we attempted to increase BC production by using a water-soluble oligosaccharide (a single sugar α-linked glucuronic acid-based oligosaccharide; SSGO) [34] as an additive to the culture medium. Based on the proposed BC metabolic pathways, we hypothesized that we could overcome the formation of the by-product (SSGO) by blocking its synthesis and therefore increase BC production by *Gluconacetobacter hansenii* PJK. The impact of SSGO addition to a static culture on the physical properties of the resulting BC were also investigated by determining various characteristics of the polymer, such as water retention, mechanical strength, and the degree of polymerization.

2. Materials and methods

2.1. Microorganism, cell culture, SSGO, and BC production

The basal medium was prepared by dissolving 10 g of glucose, 10 g of yeast extract, 7 g of peptone, 1.5 mL of acetic acid, and 0.2 g of succinate in 1 L of distilled water. The agar plates that were used to culture the *G. hansenii* PJK (KCTC 10505BP) strains were prepared from basal medium with 20 g of agar/L. The pH of the medium was adjusted to 5.0 with 1 N NaOH. The basal medium was autoclaved for 15 min at 121 °C. The *G. hansenii* PJK colonies were inoculated in 50 mL of medium in a 250 mL flask, which was shaken at 150 rpm and incubated at 30 °C for 24 h.

The SSGO was produced according to a previously described method [33]. A *G. hansenii* PJK culture broth (80 mL) was inoculated into 1.6 L of MAE medium (basal medium with 1.0%, v/v ethanol) in a 2 L jar fermenter. Fermentation was performed at 30 °C with a turbine impeller at an agitation rate of 500 rpm and an aeration rate of 1.0 vvm. The pH of the medium was maintained at 6.0. Glucose (16.8 g) was added to the 1.68 L of working volume in the fermenter at various times. The first glucose aliquot was added after 24 h of fermentation, followed by additions every 12 h for a total of 10 days. After fermentation, the culture broth was centrifuged, and the supernatant was removed and treated with 5 volumes of chilled ethanol for 1 h [33].

In a static culture, BC was produced by inoculating 5.0% of the *G. hansenii* PJK culture broth into MAE medium at 30 °C at pH 5. The BC and SSGO concentration was measured after 5, 10, and 15 days. The shaking and agitated cultures were incubated under the experimental conditions with shaking at 150 and 500 rpm, respectively, and an aeration rate of 1 vvm. The agitation speed and aeration rates were chosen to prevent the conversion of Cel^+ cells to Cel^- mutants and maximize BC production [1,25,35]. Additional experiments were performed under static conditions with the addition of 1.0 g (1.0%) of SSGO to 100 mL of MAE medium at the beginning of cultivation.

2.2. Analysis of the bacteria and BC

BC was produced in a pellet and plate forms in the shaking and static cultures, respectively. The BC was harvested from the shaking culture by centrifuging the culture broth for 20 min at 3580 × *g* and washing the pellet with distilled water; centrifugation and washing were conducted a total of three times. The BC was harvested from the static culture by simply removing the BC plate from the culture flask and washing it thoroughly with distilled water. The dry weight of BC-containing microbial cells was measured after the compound was freeze-dried at −50 °C. The BC-containing cells were autoclaved with 20 mL of 0.3 N NaOH at 121 °C for 15 min to disrupt and dissolve the microbial cells [23]. The solution was subsequently filtered through Whatman filter paper (pore size: 8 μm) with an aspirator to remove the dissolved material. The BC cake was rinsed repeatedly with distilled water until the pH of the filtrate became neutral. The BC plates obtained from the static cultures were also treated with 0.3 N NaOH for 15 min and then rinsed with distilled water. The weight of the dry BC (without microbial cells) was measured after the compound was freeze-dried at −50 °C. The difference between the weight of the dried BC containing cells and the dried BC weight after treatment with NaOH represents the dry cellular weight [22].

2.3. CFU quantification

The culture broth was diluted with saline and then spread on agar plates containing the basal medium. The plates were incubated at 30 °C until colonies formed, and the number of colony forming units (CFU) was calculated [1,24].

2.4. FE-SEM analysis

The morphology and surface topography of the BC sheets were determined by taking micrographs of platinum coated samples with a field-emission scanning electron microscope (S-4300; Hitachi Co., Japan).

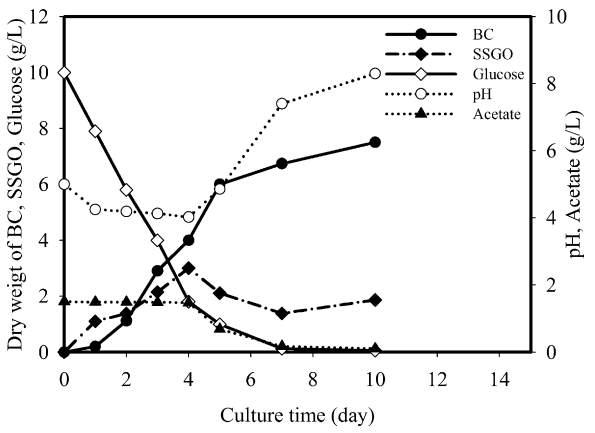

Fig. 1. A time course of BC production by *G. hansenii* PJK with MAE medium in a static cuture at 30 °C.

2.5. Gel permeation chromatography (GPC)

The average molecular weight (Mw), polydispersity index (Mw/Mn), and the degree of polymerization (DP) of the nitrated BC samples were determined using a high performance gel permeation chromatography system (GPCV 2000, Water Alliance, USA) according to a procedure previously described [36,37]. The BC samples were nitrated according to the method reported by Alexander and Michael [38]. The nitrating mixture was prepared by slowly dissolving 101 g of phosphorous pentoxide into 250 g of ice-cold nitric acid, and the solution was filtered through glass wool. BC (1 g) was mixed with 40 g of the nitrating mixture, and the mixture was incubated at 20 °C for 20 min with constant swirling. The resulting BC samples were washed with cold (10 °C) distilled water and neutralized with 5% sodium carbonate. The samples were thoroughly washed again with fresh water and boiled for 20 min in distilled water. Finally, all the samples were soaked in 50 mL of methyl alcohol for 10 min and then dried in an oven at 50 °C. The sample molecular weight was measured with a GPC with tetrahydrofuran as an eluent.

2.6. Water retention

The water retention capacity of the BC was measured with the shake method [37]. The samples were removed from the storage container using forceps, were shaken quickly twice, and were weighed. The samples were dried at room temperature for 48 h and the sample weight was subsequently measured at various times. Finally, the samples were dried for 12 h at 60 °C to completely remove all of the water. The water holding capacity was calculated as the mass of the water removed during drying per dry weight of the BC.

To determine the water release rate, the wet weight of the BC from static conditions was initially measured, and the sample was continually weighed under ambient conditions at different time intervals until a constant dry weight was achieved. The weight of the BC sample was plotted against the measurement time [37].

2.7. Mechanical strength

The tensile properties of the BC samples were measured with an Instron Universal Testing machine (Model 4465, USA) according to the procedure of the American Society for Testing and Materials (ASTM D 882) [37]. Two metal clamps were placed at either end of a 100 mm × 10 mm rectangular strip of the dried BC sample. The clamps were mounted on an Instron 4465, and both the elongation and maximum tensile load prior to compound fracture were measured.

3. Results and discussion

3.1. BC and SSGO production with various culture conditions

The production of bacterial metabolites under different culture conditions has various advantages and disadvantages. For example, static culture conditions produce a continuous sheet of BC, which would be suitable for applications in biomedical fields. In the present study, BC was produced in static, shaking, and agitated cultures.

In the static cultures, the glucose was consumed within 7 days of cultivation (Fig. 1). BC was gradually produced and reached a maximum value of 7.4 g/L on the 10th day of cultivation. SSGO

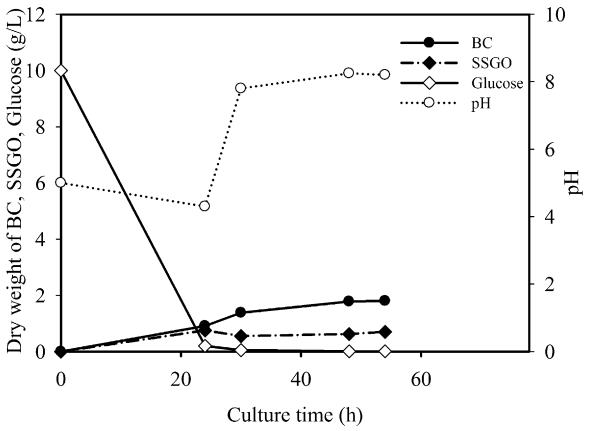

Fig. 2. A time course of BC production by *G. hansenii* PJK at 30 °C with MAE medium. Three liters of the bacterial culture was placed in a 5 L jar fermentor with 6 flat-blade turbine impellers that rotated at 500 rpm and an aeration rate of 1vvm.

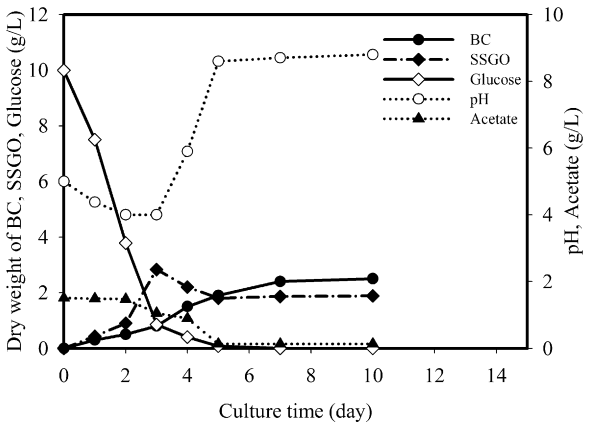

Fig. 3. A time course of BC production by *G. hansenii* PJK with MAE medium in a shaking cuture at 150 rpm and 30 °C.

was produced steadily until the 4th day of cultivation, followed by a decrease in production (Fig. 1). The pH of the culture broth decreased in association with SSGO production until the 4th day and subsequently increased to 8.4 on the 10th day of cultivation.

The agitated cultures were shaken at 500 rpm with an aeration rate of 1 vvm. In previous studies in which BC was produced with the same bacterial strain [1,39,40], 500 rpm was found to be the optimal impeller rotational speed to suppress *Cel*$^-$ mutant generation and maximize BC production. In agitated cultures, a high shear stress is generated at the tip of the impeller, and any shear stress converts *Cel*$^+$ cells to *Cel*$^-$ mutants during cultivation [1,39,40]. Because these two cell types have distinct morphologies, they can be simultaneously quantified by assessing the number of colony forming units. The *Cel*$^-$ mutants are smooth colonies while the *Cel*$^+$ are mucosal and rough. In a previous study [1] the number of *Cel*$^-$ mutants decreased as the impeller rotational speed was increased from 80 to 700 rpm. The *Cel*$^+$ cells remained imbedded in a stellate pellet of BC, whereas the *Cel*$^-$ mutants were exposed and were easily killed by the strong shear stress that was generated at the tip of impeller. However, at an impeller speed lower than 500 rpm, the number of *Cel*$^-$ mutants increased with culture incubation time until all of the cells in the fermenter were *Cel*$^-$. Moreover, the living cells in the fermenter were composed of *Cel*$^+$ cells without *Cel*$^-$ mutants with impeller speeds higher than 500 rpm [1] although only a small number of *Cel*$^+$ cells survived during cultivation. Similarly, in another study [35] 1 vvm was found to be the optimal aeration rate for avoiding *Cel*$^+$ to *Cel*$^-$ mutation; thus, we chose this aeration rate for the current study because it allowed the cells to grow and maximally produce BC.

In the agitated cultures, similar patterns of BC and SSGO production, pH variation, and glucose consumption were observed (Fig. 2) for the various culture conditions, although the scale of the culture time was decreased compared to the static cultures. The glucose was consumed within 30 h (Fig. 2), possibly due to an increased mass transfer rate. Maximal BC production was achieved in a relatively short span of time in the agitated culture; in this culture, the nutrients, cells, air, and substrates were homogeneously distributed by the continuous stirring of the medium, which produced small BC pellets [41]. Based on the general mass transfer phenomena, the smaller pellets may allow the bacterial cells inside and on the surface of the cellulose to more easily uptake nutrients and oxygen. However, the amount of both BC and SSGO produced in the agitated culture was markedly decreased compared to the static culture, as is shown in Figs. 1 and 2. The decreased BC production in the agitated culture was likely caused by conversion of the microbial cells to *Cel*$^-$ mutants, which is caused by shear stress and rapid consumption of the carbon source, glucose; however, a majority of the *Cel*$^-$ mutants were killed by maintaining the proper rotational speed of impeller. In previous studies [1], the population of *Cel*$^+$ cells increased with culture time until 30 h and subsequently decreased due to consumption of the carbon source (glucose) after 24 h of cultivation. As is shown in Table 1, the dry cell weight of the agitated culture increased rapidly to 2.81 and 3.21 g/L after 24 and 48 h of cultivation, respectively. However, the live cell density (CFU) decreased with culture time. In contrast, the dry cell weight in the static culture increased gradually to 1.01, 2.00, and 2.22 g/L at 5, 10, and 15 days of cultivation, respectively. The live cell population was maintained for more than 10 days, although it decreased rapidly after 1 day in the agitated culture, as shown in Table 1. Therefore, the proportion of *Cel*$^+$ cells is a better indicator for BC production yield than the simple dry cellular weight. The decreased BC production in the shaking culture may have been caused by bacterial conversion to *Cel*$^-$ mutants (Fig. 3), although some pellicle-type BC can be produced from MAE medium containing 1% ethanol [24]. However, the amount of SSGO produced in the shaking culture was similar to the amount that was produced in the static culture (Figs. 1 and 3).

The pH of the medium decreased at the beginning of the culture and subsequently increased. A similar trend was previously reported, and the researchers hypothesized that some glucose was converted into gluconate by membrane-bound glucose dehydrogenase, which lowered the pH of the medium [42]. Subsequently, gluconate metabolism by the microbial cells increases the pH of the medium [43]. Yunoki et al. performed ^{13}C NMR to show that ethanol was not utilized as a carbon source for BC production; the ethanol was oxidized to acetic acid [44], which in turn is also oxidized [40]. As is shown in Figs. 1 and 3, the acetate concentration rapidly decreased on the 5th day of static culture and 3rd day of shaking culture. The pH of the culture media increased rapidly with the consumption of acetate. The culture media pH increased suddenly around the time that SSGO was metabolized, although BC was steadily produced in all three culture conditions (static, agitated, and shaking). SSGO may have decreased during the later cultivation period because the microbial cells consumed the SSGO that was produced during the previous culture period.

3.2. Role of additional SSGO in BC production

Biochemical pathways allow us to understand the mechanism(s) for BC synthesis, and thus hypothesize ways to modulate BC production. For example, previous studies have shown that controlling the formation of side products can increase BC production [31,44]. In the present study, biochemical pathways for the pro-

Table 1
Dry cell weight and CFU values obtained during the BC production by *G. hansenii* in static culture, using MAE medium and MAE medium containing 10 g/L SSGO, and in agitation culture using MAE medium.

Culture time (day)	Static				Culture time (day)	Agitation	
	Cells dry weight (g/L)		CFU/mL			Cells dry weight (g/L)	CFU/mL
	With SSGO	Without SSGO	With SSGO	Without SSGO		Without SSGO	Without SSGO
5	1.94 ± 0.15	1.01 ± 0.11	$2.5 \times 10^5 \pm 2.8 \times 10^4$	$1.3 \times 10^5 \pm 1.4 \times 10^4$	1	2.81 ± 0.17	$1.3 \times 10^7 \pm 1.5 \times 10^6$
10	3.25 ± 0.18	2.00 ± 0.13	$1.2 \times 10^5 \pm 2.1 \times 10^4$	$7.4 \times 10^4 \pm 3.5 \times 10^3$	2	3.21 ± 0.18	$9.3 \times 10^3 \pm 4.1 \times 10^2$
15	3.43 ± 0.17	2.22 ± 0.12	0	0			

duction of BC and SSGO were delineated from data in previous reports [27,45–47], as is shown in Fig. 4. SSGO is produced as a by-product during BC production by *G. hansenii* [34]. The enzymes cellulose synthase and UDP-glucose transferase produce BC and acetan, respectively. UDP-glucose is the precursor for the synthesis of both products. The inhibition of UDP-glucose transferase inhibits acetan production and hence, increases BC production [31]. Naritomi et al. [46] reported that ethanol supplementation to the culture media markedly increased BC production. Ethanol was converted to acetate, which was utilized by the TCA cycle to ultimately produce CO_2, H_2O, and ATP. This process increased the ATP content of the viable cells, which affected the activity of the enzymes involved in the BC synthesis pathway [46]. As a result, the activity of glucose-6-phospahte dehydrogenase decreased by greater than 50%, which inhibited the pentose phosphate pathway and increased BC production [46]. However, several researchers have reported that ethanol could not be used as a carbon source for BC production [44,46]. Glucose was not converted to pyruvate through glycolysis because the acetic acid bacteria did not synthesize the crucial enzyme of glycolysis, phosphofructokinase [48]. Winehouse reported that cellulose synthesis was associated with the catabolic process of oxidation and that it consumed as much as 10% of the energy derived from catabolic reactions [49]. Most of cellulose producing acetic acid bacteria can convert glucose to gluconate using membrane-bound glucose dehydrogenase. Gluconate is incorporated intracellularly and the substrate returns back for BC synthesis through Entner-Doudorouff (ED) pathway and pentose phosphate (PP) pathway. Only a small portion of the triose phosphate formed via ED and PP pathway is converted into pyruvate and most of it is recycled into hexose phosphate via aldose [50,51].

In the present study, 1.0% (w/v) of SSGO was added to the MAE medium to control SSGO formation by blocking its synthesis and therefore increase BC production. There was no net production of SSGO during BC production (Fig. 5), although BC production increased significantly. This significant increase in BC production may have been caused by inhibition of the enzyme that produces SSGO. The rate of glucose consumption increased, and almost all of the glucose was consumed within 3 days of cultivation (Fig. 5). This

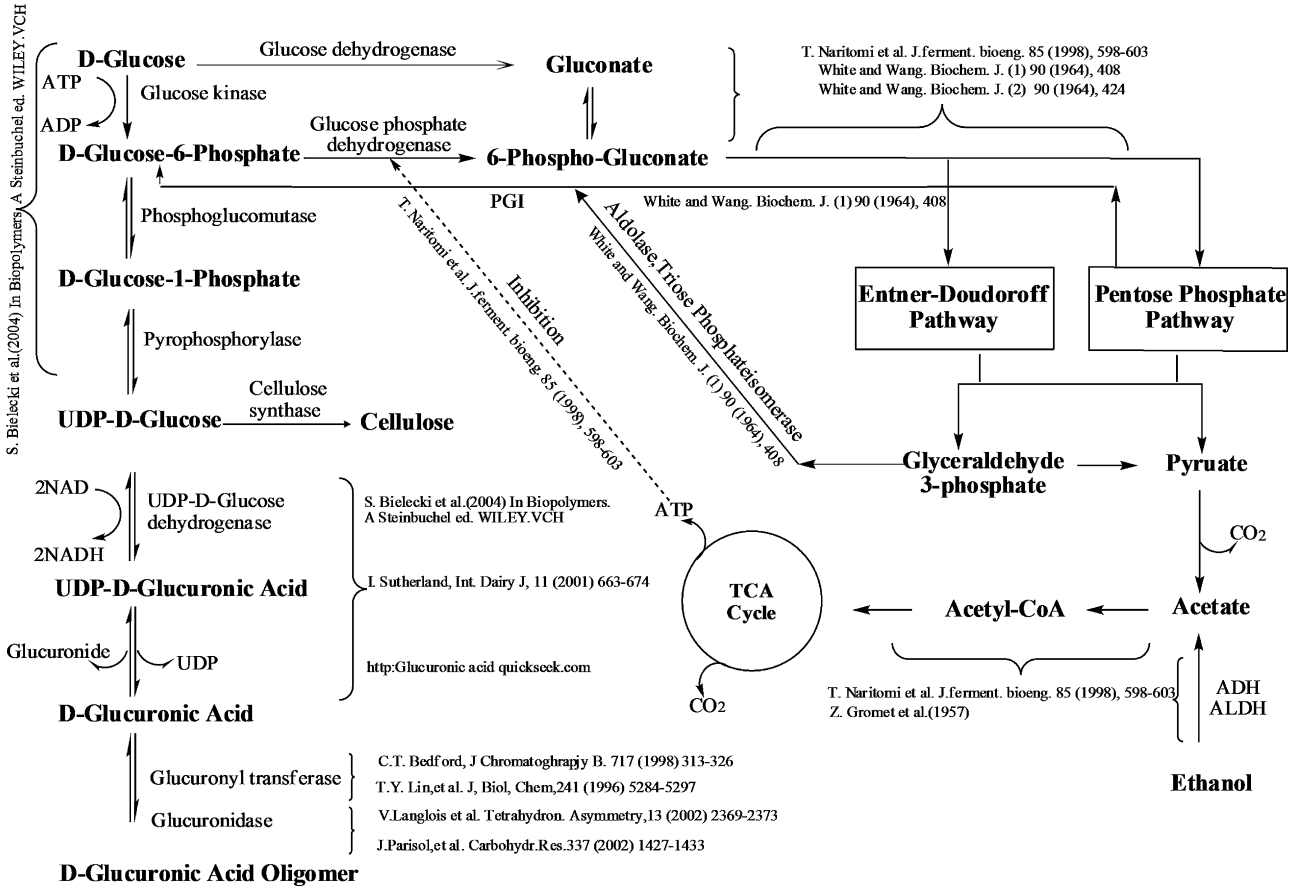

Fig. 4. Proposed biochemical pathways for the production of BC and glucuronic acid oligomers based on previous reports.

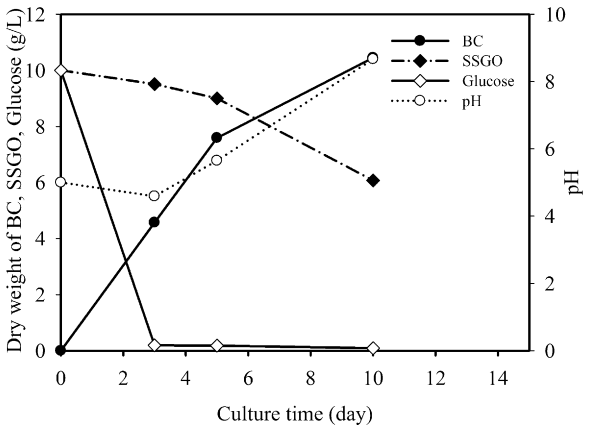

Fig. 5. A time course of BC production by *G. hansenii* PJK in MAE medium containing 10 g/L SSGO in a static cuture at 30 °C.

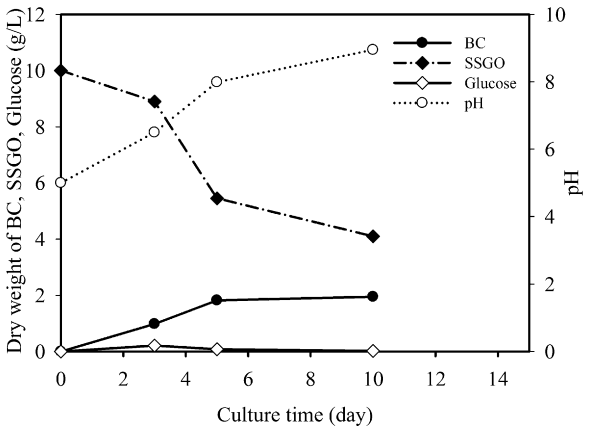

Fig. 6. A time course of BC production by *G. hansenii* PJK in MAE medium containing 10 g/L SSGO instead of glucose in a static cuture at 30 °C.

rapid consumption of glucose resulted in cell growth. The cell dry weights and the live cell density (CFU) measured on the 5th day of cultivation were nearly two-fold higher than for the MAE medium (Table 1). These observations indicate that glucose is the preferred substrate for cell growth and BC production.

As is shown in Fig. 5, SSGO was consumed during cultivation, and the initial amount of SSGO was depleted by 40% on the 10th day of cultivation. The amount of BC produced by day 10 reached 10.5 g/L in the MAE medium with 1% SSGO, but only 7.4 g/L in the MAE medium during the same time period (Fig. 1). The impact of SSGO addition on BC production was further investigated by culturing microbial cells in MAE medium without glucose and in MAE medium containing 10 g/L SSGO instead of glucose. A small amount of BC (0.8 g/L) was produced in the MAE medium without glucose by day 10. The amount of SSGO produced during BC production reached 1 g/L on day 3 and decreased to 0.75 g/L on day 10 of cultivation. The pH profile was similar to the data for BC production with MAE medium. As is shown in Fig. 6, the amount of BC produced with MAE medium containing 10 g/L SSGO instead of glucose increased steadily and reached 2 g/L on the 10th day of cultivation, at which time 60% of the SSGO was consumed. Thus, the BC production yield with MAE medium containing SSGO instead of glucose was 0.33 g BC/g SSGO, which was only half of the yield obtained from MAE medium (0.7 g BC/g glucose). However, a small amount of glucose (0.21 ± 0.03 g/L) was produced on the 3rd day of cultivation using the MAE medium containing SSGO instead of glucose. The pH increased and reached 8.65 on the 10th day, and there was no further change in pH during cultivation. Because SSGO is comprised of glucuronic acid oligomers [33], which are acidic in nature [52], a decrease in the SSGO concentration may have caused the increase in pH that was observed.

The amount of SSGO consumed in the MAE medium with 1% SSGO appeared to correspond to an increase in BC production. Previous studies have suggested that glucuronic acid production from UDP-glucose is a reversible process [27] (Fig. 4). Hence, a small amount of SSGO may have been converted to UDP-glucose and utilized in BC production as glucose became limiting.

3.3. Effect of SSGO addition to the culture media on the physico-mechanical properties of BC

The composition of the culture medium and fermentation conditions affects various properties of BC [37]. Therefore, we assessed the physical properties of the BC produced under our experimental conditions. These important properties impact the industrial and biomedical applications.

The FE-SEM micrographs shown in Fig. 7 show the morphology of the BC sheets that were prepared in the MAE medium with and without SSGO. All of the BC sheets consisted of thread-like structures or fibrils. The BC obtained with SSGO addition contains thicker fibrils that are less dense than those made in the absence of SSGO. The GPC results in Table 2 show that the number average molecular weight (Mn), the weightaverage molecular weight, and the degree of polymerization were increased in the control BC compared to the BC obtained from the MAE medium containing SSGO. However, the polydispersity index (PDI) of the control BC was lower compared to the MAE + SSGO BC. The Mn, Mw, and DP were likely decreased for the MAE + SSGO BC because SSGO is an emulsifier [34] that causes the dispersion of BC particles or constituents [19,41]. This disper-

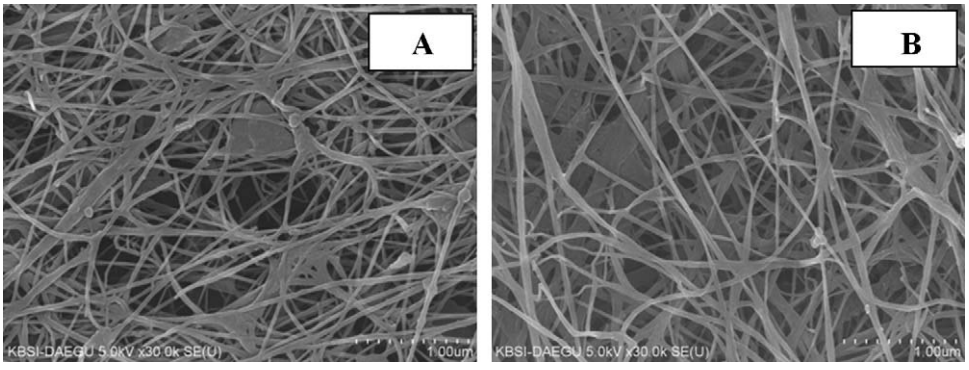

Fig. 7. FE-SEM micrographs of bacterial cellulose produced by *G. hansenii* for 10 days in static cultivation using (A) MAE medium and (B) MAE medium containing 10 g/L SSGO.

Table 2
Mechanical strength, molecular weight and its distribution for bacterial cellulose produced by *G. hansenii* for 10 days in static cultivation using MAE medium and MAE medium containing 10 g/L SSOG.

BC sample	Mn	Mw	Mp	PDI	DP	Max. tensile stress (MPa)	Young's modulus (MPa)
SSOG 0.0%	1,559,000	2,096,000	5,129,000	1.34	9622	2.59	245.4
SSOG 1.0%	1,356,000	1,935,000	3,418,000	1.43	8368	12.62	383.4

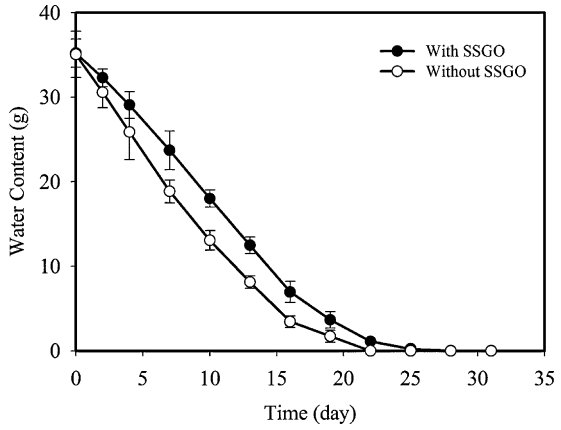

Fig. 8. The water release rate for BC produced by *G. hansenii* for 10 days in static cultivation using (A) MAE medium and (B) MAE medium containing 10 g/L SSGO. All data points are the average of triplicate analyses.

sion may have affected bond formation between the monomers, resulting in a decreased fibril length and short chains/ribbons of BC.

We assessed the tensile strength of the BC sheets to determine whether the addition of SSGO impacted the mechanical properties of BC. The BC sheets prepared in the MAE medium containing SSGO had a higher tensile strength and elongation at the break compared to the control (Table 2). The MAE + SSGO BC had thicker fibers and a higher polydispersity, but a lower DP, compared to the control. The thicker fibers and higher polydispersity may be directly related to the superior mechanical properties.

The slow release of water from BC makes it suitable for biomedical applications, such as wound dressings [8,53]. The water holding capacity of the BC produced in the MAE medium containing SSGO was nearly the same as that of the BC produced in the MAE medium (35.2 times its dry weight). However, water release from the BC sheets obtained in the MAE medium containing SSGO was slower compared to the control (Fig. 8). Because of its slower rate of water release, the final dry weight of the BC sheets produced in the MAE medium containing SSGO was reached after 28 days, while the final dry weight of the BC obtained in MAE medium was reached after 22 days. The thicker fibrils and higher polydispersity of the BC produced in the MAE medium containing SSGO (Fig. 7 and Table 2) may have sandwiched the water molecules and prevented them from escaping at a high speed from BC membrane, as has been reported previously [37]. Several biomedical applications of BC require a slow release of water [8,53]; thus, in this context, the BC produced with the MAE medium containing SSGO seems to be superior to the BC produced with the MAE medium alone.

4. Conclusion

In the current study, we assessed the suitability of SSGO as a carbon source and its impact on BC production in detail. Moreover, we studied the impact of SSGO media supplementation on various physical characteristics of the produced BC. SSGO addition to the MAE medium at the beginning of cultivation increased BC production by inhibiting glucuronic acid oligomer synthesis. Moreover, SSGO could be used as an alternative carbon source to glucose. Finally, SSGO addition enhanced several physical characteristics of the BC. In conclusion, our study shows that SSGO can inhibit glucuronic acid oligomer synthesis during BC production and improve the physical characteristics of BC.

Acknowledgements

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